JOURNAL OF CLINICAL MICROBIOLOGY, July 1989, p. 1601–1608
0095-1137/89/071601-08$02.00/0

Vol. 27, No. 7

Francisella philomiragia comb. nov. (Formerly *Yersinia philomiragia*) and *Francisella tularensis* Biogroup Novicida (Formerly *Francisella novicida*) Associated with Human Disease

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Received 3 October 1988/Accepted 10 March 1989

Over a 12-year period, 16 human strains of a gram-negative, catalase-positive, halophilic, aerobic, nonmotile, small coccoid bacterium were received for identification. On the bases of biochemical characteristics and cellular fatty acid profiles, 14 of these strains were similar to the "Philomiragia" bacterium (*Yersinia philomiragia*, species incertae sedis). Additional characteristics were growth on Thayer-Martin agar but no growth or sparse, delayed growth on MacConkey agar; oxidase positive; acid production, often weak and delayed, from D-glucose, sucrose, and maltose; urease negative; no reduction of nitrates; and H_2S produced but often delayed in triple sugar iron agar. Both the human isolates and the "Philomiragia" bacterium contained $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ ω9c, $C_{18:0}$, 3-OH $C_{18:0}$, $C_{22:0}$, and $C_{24:1}$ as major cellular fatty acids and ubiquinone eight (Q8) as the major isoprenoid quinone. These cellular acids in these relative amounts have been found previously only in *Francisella tularensis* and *Francisella novicida*, suggesting a relationship between the "Philomiragia" bacterium and *Francisella* species. Of the 14 human "Philomiragia"-like isolates, 9 were from blood, 3 were from lung biopsies or pleural fluid, and one each was from peritoneal fluid and cerebrospinal fluid. DNA relatedness studies (hydroxyapatite method, 50 and 65°C) showed that these 14 strains were a single group that was the same species as the "Philomiragia" bacterium. Two other human strains were oxidase negative and H_2S negative. They formed a single DNA relatedness group that was indistinguishable from the type strains of both *F. tularensis* and *F. novicida*. DNA relatedness of "Philomiragia" bacterium type and other strains to strains of *F. novicida* and *F. tularensis*, including the type strains, was 35 to 46%. One of the two *F. novicida*- and *F. tularensis*-like strains was isolated from blood, and the other was isolated from a cervical lymph node. On the basis of these findings, we propose transferring *Y. philomiragia* from the genus *Yersinia* to the genus *Francisella* as *Francisella philomiragia* comb. nov. Having confirmed that *F. novicida* and *F. tularensis* are the same species and having shown that *F. novicida* is pathogenic for humans, we further propose eliminating the species *F. novicida* and demoting it to a biogroup of *F. tularensis*.

During the past 12 years the Special Bacterial Reference Laboratory of the Centers for Disease Control received for identification 16 human isolates of gram-negative, catalase-positive, halophilic, aerobic, small coccoid bacteria. Recently it was recognized that these isolates were similar to the "Philomiragia" bacterium (*Yersinia philomiragia*), which had not previously been associated with human disease.

The name *Y. philomiragia* was proposed in 1969 by Jensen et al. (13) for a small gram-negative bacillus first isolated in 1959 from a dying muskrat found in a marshy area in Utah. The description of this organism was based on the study of this strain and four other strains isolated from water samples from the same area in 1960. No additional isolates of *Y. philomiragia* have been reported. This new bacterium was placed in the genus *Yersinia* because of its DNA relatedness to *Yersinia pestis* (24%), reported in 1966 by Ritter and Gerloff (25), and its morphologic resemblance to *Y. pestis* in animal tissues. In 1974, O'Hara et al. (20) noted serologic cross-reactions between *Y. philomiragia* and species of the genera *Francisella* and *Brucella* and recommended that its taxonomic status be reconsidered. In 1980 Ursing et al. (30) studied four *Y. philomiragia* strains, including the type strain, but did not demonstrate any significant DNA relatedness between *Y. philomiragia* and other *Yersinia* species, other species of *Enterobacteriaceae*, or *Pasteurella multocida*. DNA relatedness between *Y. philomiragia* and *Francisella* species was not determined. These authors suggested that, pending further study, this bacterium be referred to as the "Philomiragia" bacterium. *Y. philomiragia* appears on the Approved Lists of Bacterial Names (28); however, it is currently considered a species incertae sedis which is misclassified in the genus *Yersinia* (1).

Francisella tularensis, the etiologic agent of tularemia, was first described in 1912 (17). It was originally in the genus *Bacterium* and was subsequently placed in the genus *Pasteurella*, although it was also placed in the genus *Brucella* on a provisional basis (22, 31). The proposal to form the genus *Francisella* with *F. tularensis* as its single species was made in 1947 (8); however, *Pasteurella tularensis* appeared in the 7th edition of *Bergey's Manual of Determinative Bacteriology* in 1957 (3), and *Francisella* was not generally accepted until the mid-to-late 1960s. *Francisella novicida* was isolated in 1951 from a water sample in Utah (15). In 1955, it was classified in the genus *Pasteurella* (15), and it was transferred to the genus *Francisella* in 1959 (21). Only one strain has been reported.

F. novicida and *F. tularensis* share many antigenic, bio-

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chemical, and genetic similarities, and these species show a strong serologic cross-reaction (23). High-frequency transformation occurs between species (29), and DNA relatedness between the species is 78 to 97% (25). *F. novicida*, but not *F. tularensis*, was reported to produce acid from sucrose (10). *F. tularensis* is somewhat more fastidious and in general is more virulent than *F. novicida* (10), but it must be noted that the data on *F. novicida* are from only one strain.

Our interest in these organisms was renewed when we received several "Philomiragia" bacterium-like cultures from humans. We decided to perform a study to determine the possible relationship of the "Philomiragia" bacterium and the 16 human clinical isolates that resembled the "Philomiragia" bacterium. The biochemical characteristics, cellular fatty acid compositions, and DNA relatedness of the "Philomiragia" bacterium-like human isolates, the "Philomiragia" bacterium, *F. novicida*, and *F. tularensis* were investigated. On the basis of these data we determined that 14 of the human strains were related at the species level to the "Philomiragia" bacterium, which was shown to belong in the genus *Francisella*. The two remaining human strains were shown to be *F. novicida*, which we confirmed to be the same genetic species as *F. tularensis*. We therefore propose the name *Francisella philomiragia* comb. nov. for the "Philomiragia" bacterium and propose that *F. novicida* be considered a biogroup of *F. tularensis*.

(A portion of this work has been presented [D. G. Hollis, C. W. Moss, and R. E. Weaver, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C272, p. 373].)

MATERIALS AND METHODS

Bacterial strains. This study began with 16 human isolates that resembled the "Philomiragia" bacterium. On the basis of biochemical characteristics, two of these strains, D9876 and F6168, were similar to *F. novicida* and the other 14 strains were similar to *Y. philomiragia*. These strains and information received with the cultures are given in Table 1. In addition, the following strains were used in the study: "Philomiragia" bacterium (*Y. philomiragia*) ATCC 25015^{T}, ATCC 25016, KC1394 (derived from ATCC 25017), and KC1395 (derived from ATCC 25018); *F. novicida* KC666^{T} (derived from Utah 112^{T}); and *F. tularensis* KC1482^{T} (derived from ATCC 6223^{T}), KC1458 (SCHU-4 obtained from Ft. Detrick, Md.), E3526 (rabbit blood from Massachusetts), E6702 (thoracentesis fluid from Iowa), and F6292 (cervical node from Washington).

Morphology and biochemical tests. The cultures were identified by using the conventional cultural and biochemical tests and methods used in the Special Bacterial Reference Laboratory of the Centers for Disease Control (6) as listed in Table 2. Incubation temperature was 35°C. The Kovacs modification of the oxidase test was done on bacterial growth from an 18- to 24-h blood agar plate (heart infusion agar [HIA] with 5% rabbit blood [HIAB]). The development of a light- to dark-blue color within 10 s indicated a positive reaction. HIA slants were used for growth temperature tests. Three methods were used to detect indole production at 48 h, 4 days, and 7 days: (i) tryptone broth tested with Ehrlich-Boehme reagent after xylene extraction (6); (ii) heart infusion broth tested with Ehrlich-Boehme reagent after xylene extraction (6); and (iii) peptone water tested with Kovacs reagent (9).

Fatty acid analysis. Cells for fatty acid and isoprenoid quinone analysis were obtained by inoculating strains onto HIAB plates. The plates were incubated at 35°C for 24 h for cellular fatty acid analysis and for 48 h for quinone analysis. The cells were processed by the method previously described (7).

TABLE 1. "Philomiragia" bacterium-like and *F. novicida*-like strains

Bacterial type and culture no.	Date received (mo-day-yr)	Geographic source	Clinical source
"Philomiragia" bacterium-like			
D2204	2-04-75	California	Lung biopsy
D7533	11-08-76	Colorado	Pleural fluid
E384	10-04-77	New York City	Blood
E3596	10-25-78	California	Blood
E6588[a]	8-30-79	Switzerland	Blood, bone marrow, and ascitic fluid
E7485	2-27-80	Pennsylvania	Blood
E8869	8-13-80	Connecticut	Blood
E9088	9-18-80	Connecticut	Blood
E9923	1-30-81	New York City	Blood
F1853	11-20-81	California	Lung biopsy
F6055	8-30-84	New Mexico	Peritoneal fluid
F6820	6-04-85	Virginia	Blood
F9017	10-23-86	New York	Pericardial fluid and blood
F9693	3-16-86	Massachusetts	Cerebrospinal fluid
F. novicida-like			
D9876	7-21-77	Louisiana	Cervical lymph node
F6168	10-15-84	California	Blood

[a] Case reported (see reference 27).

The fatty acid methyl esters were analyzed on a fused-silica capillary column (50 m by 0.32 mm) with cross-linked methyl silicone (OV-101) as the stationary phase (Hewlett-Packard Co., Avondale, Pa.). The column was installed in a Hewlett-Packard 5880 gas chromatograph equipped with a flame ionization detector coupled to a level 4 integrator and data system. The operating parameters of the instrument were as follows: injector temperature, 250°C; detector temperature, 300°C; column temperature, programmed from 140 to 275°C at 6.5°C/min and maintained at 275°C for 16 min before recycling back to 140°C. The fatty acid methyl esters were identifed by comparing retention times to reference standards (Supelco, Inc., Bellefonte, Pa.), and this identity was confirmed by trifluoracetylation, hydrogenation, and mass spectrometry (14).

Determination of isoprenoid quinones. Cells from five HIAB plates were hydrolyzed by adding 0.2 ml of 50% aqueous KOH and 3 ml of 1% pyrogallol in methanol and heating them at 100°C for 10 min. After being cooled to room temperature, the quinones were extracted and examined by reverse-phase high-performance liquid chromatography as described previously (7, 18). Tentative identification was established by comparing retention times with authentic standards supplied by Hoffmann-La Roche Co. (Basel, Switzerland). Identification was confirmed by collecting fractions from reverse-phase high-performance liquid chromatography followed by analysis with both electron impact and chemical ionization mass spectrometry (7, 18).

Antimicrobial agent susceptibility tests. Antimicrobial agent susceptibility was determined by using the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (19), except the

TABLE 2. Biochemical characteristics of "Philomiragia" bacterium-like, *Y. philomiragia*, *F. novicida*-like, and *F. novicida* strains[a]

Test or substrate	"Philomiragia" bacterium-like strains (14 human isolates)	*Y. philomiragia* (2 strains)	*F. novicida* Utah 112[T b]	*F. novicida*-like strains (2 human isolates)
Oxidase, Kovacs modification	100	100	–	0
Growth on:				
MacConkey agar	14W (14W)	50W	(W+)	(50W)
Thayer-Martin agar	100	100	+	100
Triple sugar iron agar				
Acid slant	0	0	–	0
Acid butt	0	0	–	0
H_2S butt or slant	64W (36)	(100W)	–	0
Catalase	36, 57W	100	+	50W
Gelatin hydrolysis	(79)	(50)	–	0
Growth in:				
Nutrient broth, 0% NaCl	0	0	–	0
Nutrient broth, 6% NaCl	93	(50)	–	100
Nutrient broth, 8% NaCl	7	0	–	0
Acid production from:				
Difco OF base				
D-Glucose	57W (43W)	100W	(W+)	(100W)
D-Xylose	0	0	–	0
D-Mannitol	0	0	–	0
Lactose	0	0	–	0
Sucrose	57W (43W)	100W	(W+)	(100W)
Maltose	57W (43W)	100W	–	(100W)
Peptone broth base, D-glucose	(21, 43W)	(50W)	(+)	(100)
Growth at:				
25°C	100	100	+	100
35°C	100	100	+	100
42°C	14	50	+	50

[a] Tests results are given as percent positive after 24 to 48 h or as negative (–) or positive (+). ( ), Delayed 3 to 7 days; W, weak reaction. The following tests or reactions were negative with all strains tested in this study: Gram stain, anaerobic growth, motility, Christensen urea, nitrate reduction, indole, esculin hydrolysis, Simmons citrate, litmus milk, L-lysine and L-ornithine decarboxylases, and L-arginine dihydrolase.

[b] Type strain.

incubation was in a candle extinction jar. β-Lactamase was assayed as described by Schoenknecht et al. (26).

DNA studies. Guanine-plus-cytosine (G+C) content was determined spectrophotometrically by thermal denaturation (16). The preparation and purification of DNA and the conditions used to determine DNA relatedness by the hydroxyapatite method have been described previously (4). DNA from the "Philomiragia" bacterium (*Y. philomiragia*) ATCC 25015^T and *F. tularensis* KC1482^T (ATCC 6223^T) were labeled in vitro with [^{32}P]dCTP provided in a nick-translation reagent kit (Bethesda Research Laboratories, Gaithersburg, Md.) (5). Hybridization reactions were done at 50°C (optimal reassociation) and 65°C (stringent reassociation) (4).

RESULTS

Morphology and biochemical tests. At 24 h, colonies of the "Philomiragia" bacterium-like strains and the *F. novicida*-like strains on HIAB at 35°C were punctate to 0.5 mm in diameter, convex with entire edges, semitranslucent, smooth, glossy, and somewhat butyrous in consistency. Colonies of the "Philomiragia" bacterium-like strains tended to be slightly stringy. By 36 to 48 h, the colonies were usually 1 to 2 mm in diameter. The "Philomiragia" bacterium-like strains often produced a lavender-green coloration and a clearing of the blood agar under the confluent growth. From growth on HIA at 24 h, the bacterial cells were somewhat pale-staining, gram-negative, slightly small to small coccoid organisms, sometimes with slight pleomorphism.

All strains grew at 35 and 25°C on HIA. Three "Philomiragia" bacterium-like strains grew best at 25°C; only two strains grew at 42°C. Both *F. novicida*-like strains grew best at 35°C, and only one grew at 42°C. No strains grew on HIAB plates incubated anaerobically for 3 days. All strains grew on Thayer-Martin selective medium, buffered charcoal-yeast extract agar, and buffered charcoal-yeast extract agar without cysteine at 35°C in a candle jar or ambient atmosphere.

Table 2 shows the results of the biochemical tests of the 14 human "Philomiragia" bacterium-like isolates along with those of two reference strains of *Y. philomiragia*, two human *F. novicida*-like strains, and the type strain of *F. novicida*. The Kovacs oxidase reaction was positive for all the "Philomiragia" bacterium-like strains and negative for the *F. novicida*-like strains. The Kovacs modification is a more sensitive procedure for detecting oxidase (6). Weak or delayed H_2S production in the butt or on the slant of triple

TABLE 3. Cellular fatty acid composition of "Philomiragia" bacterium-like, *F. novicida*-like, and *F. tularensis* strains

Fatty acid[a]	% (range) of total fatty acids[b] in:		
	"Philomiragia" bacterium-like strains (16 strains)[c]	*F. tularensis* (14 strains)	*F. novicida*-like strains (3 strains)[d]
$C_{10:0}$	13 (10–13)	30 (10–58)	10 (9–11)
2-OH $C_{10:0}$	— (0–1)	1 (0–4)	5 (4–6)
$C_{12:0}$	— (0–1)	— (0–1)	— (0–1)
$C_{14:0}$	16 (8–25)	11 (4–15)	12 (12–13)
$C_{16:0}$ ald	2 (0–5)	1 (0–4)	2 (1–2)
$C_{16:0}$	9 (6–15)	10 (5–13)	18 (17–19)
3-OH $C_{16:0}$	3 (2–4)	3 (0–6)	2 (2–3)
$C_{18:2}$	2 (1–3)	2 (0–3)	1 (1–2)
$C_{18:1}$ ω9c	12 (9–17)	7 (4–8)	9 (8–10)
$C_{18:0}$	9 (6–13)	3 (1–4)	8 (6–9)
3-OH $C_{18:0}$	9 (6–11)	12 (7–24)	10 (8–12)
$C_{20:1}$	— (0–1)	— (0–1)	1 (1–2)
$C_{20:0}$	3 (2–4)	1 (0–3)	2 (2–3)
$C_{22:1}$	1 (1–2)	1 (0–2)	2 (1–3)
$C_{22:0}$	6 (4–8)	5 (3–10)	5 (4–6)
$C_{24:1}$	9 (5–14)	5 (0–8)	7 (6–8)
$C_{24:0}$	4 (0–9)	5 (0–10)	3 (2–3)
$C_{26:1}$	— (0–2)	— (0–1)	— (0–1)
$C_{26:0}$	— (0–1)	— (0–1)	— (0–1)

[a] Shorthand designation: the number before the colon indicates number of carbon atoms, and the number after the colon indicates number of double bonds. The symbols 2-OH and 3-OH indicate an hydroxy group at the 2- and 3- carbons, respectively. ald, Aldehyde.
[b] Values are arithmetic means. —, Not detected or less than 0.8%.
[c] Includes two *Y. philomiragia* strains.
[d] Includes *F. novicida* type strain.

sugar iron agar was observed with all "Philomiragia" bacterium-like strains, while none was observed with the *F. novicida*-like strains. *Y. philomiragia* was reported previously as giving a weak indole reaction (30); however, we were unable to detect indole production by any of the three methods used. None of the strains grew in nutrient broth without NaCl, while growth in nutrient broth with 6% added NaCl varied among strains within each species. Gelatin hydrolysis was negative with *F. novicida*-like strains but was often positive with "Philomiragia" bacterium-like strains. Motility was not observed in motility medium or in a wet preparation with any of the strains.

***F. tularensis* direct fluorescent-antibody test.** Because the cellular morphology of these organisms was somewhat reminiscent of *F. tularensis*, a fluorescent-antibody test was kindly done on the 16 human strains and the type strain of *F. novicida* by W. F. Bibb, Immunology Laboratory, Meningitis and Special Pathogens Branch, Centers for Disease Control. Three "Philomiragia" bacterium-like strains (D7533, E3596, and E6588) and the type strain of *F. novicida* stained 3 to 4+ with the *F. tularensis* conjugate.

Fatty acid analysis. Quantitative cellular fatty acid composition data for the "Philomiragia" bacterium-like and *Y. philomiragia* strains, the *F. novicida*-like and *F. novicida* strains, and the *F. tularensis* strains are presented in Table 3. The overall fatty acid profile of the "Philomiragia" bacterium-like strains is strikingly similar to that of *F. tularensis* as reported by Jantzen et al. (12) and previously recorded in our laboratory. Both species, as well as *F. novicida*-like strains, are characterized by the presence of long-chain saturated and monounsaturated C_{18} to C_{26} acids, relatively large amounts of saturated even-chain acids ($C_{10:0}$, $C_{14:0}$, and $C_{16:0}$), and two long-chain hydroxy acids (3-OH $C_{16:0}$ and 3-OH $C_{18:0}$). The presence and relative amounts of these acids constitute a fatty acid profile which in our experience is unique for the genus *Francisella* (including *Y. philomiragia*). Additional unusual features compared with other gram-negative bacteria are the absence of a monounsaturated 16-carbon acid in all strains, the absence of dodecanoic acid ($C_{12:0}$) in most strains, and the presence in most strains of small amounts (1 to 2%) of a 16-carbon aldehyde (Table 3).

No distinguishing differences in fatty acid composition were observed among "Philomiragia" bacterium-like, *F. tularensis*, and *F. novicida*-like strains. In general, *F. tularensis* contained larger amounts of decanoic acid ($C_{10:0}$) than the other organisms, but this feature is not useful for differentiation because of the overlap in relative amounts of this acid among strains (Table 3). The *F. novicida*-like strains contained small amounts of 2-hydroxydecanoic acid (2-OH $C_{10:0}$), while only about 50% of *Y. philomiragia*, "Philomiragia" bacterium-like, and *F. tularensis* strains contained more than trace amounts (>0.8%) of this acid. These results differ from those of Jantzen et al. (12), who reported small amounts (1 to 5%) of 2-OH $C_{10:0}$ in each of nine *F. tularensis* strains. Jantzen et al. (12) also reported trace amounts (0 to 0.7%) of $C_{10:0}$, whereas each of our strains contained large (9 to 58%) amounts of this acid (Table 3). We also observed smaller amounts of 3-OH $C_{18:0}$ than was reported by Jantzen et al. (12). However, even with these observed quantitative differences, which may be due to slight differences in methods or growth media, the genus *Francisella* was readily identified by its unique fatty acid profile.

Determination of isoprenoid quinones. All "Philomiragia" bacterium-like, *F. novicida*-like, and *F. tularensis* strains contained a ubiquinone (Q) with eight isoprene units (Q8) as their major isoprenologs. Small amounts of Q7 and Q9 were also detected, but no menaquinones were present. The identity of Q8 in these species was firmly established by mass spectrometry, which showed a base peak at *m/e* 235 and a large peak at *m/e* 726 corresponding to the molecular ion. The molecular ion was verified by chemical ionization spectra, which gave intense M + 1 ions at the expected mass value of *m/e* 727.

Antimicrobial susceptibilities. All strains (14 "Philomiragia" bacterium-like strains, two *Y. philomiragia* strains, two *F. novicida*-like strains, and the type strain of *F. novicida*) were susceptible to all the quinolones (nalidixic acid, norfloxacin, and ciprofloxacin), all the aminoglycosides (streptomycin, gentamicin, tobramycin, and amikacin), tetracycline, chloramphenicol, moxalactam, cefotaxime, and cefoxitin. Sixty-nine percent of the strains were susceptible to amoxicillin plus clavulanic acid, 42% were susceptible to rifampin, and 26% were susceptible to cephalothin. Seventy-five percent of the strains were moderately susceptible to erythromycin. All isolates were resistant to ampicillin, and all were β-lactamase positive. No distinct differences in the antimicrobial susceptibility patterns of the two groups of organisms could be detected.

DNA studies. DNAs from the type strains of *F. tularensis*, *F. novicida*, and *Y. philomiragia* as well as from two other *Y. philomiragia* strains contained 34 mol% G+C. The G+C content of DNAs from four "Philomiragia" bacterium-like strains was 33 to 34 mol%. Because of these relatively low G+C values, 50°C was chosen as the temperature for optimal DNA reassociation and 65°C was used as a stringent criterion for DNA reassociation.

Labeled DNA from *Y. philomiragia* ATCC 25015^T was 81 to 86% (average, 84%) related to unlabeled DNAs from three other *Y. philomiragia* strains in 50°C reactions (Table 4).

TABLE 4. DNA relatedness of *Y. philomiragia* ATCC 25015^T to "Philomiragia" bacterium-like, *F. novicida*-like, and *Francisella* strains

Source of unlabeled DNA	% Relatedness (50°C)	% Divergence[a]	% Relatedness (65°C)
Y. philomiragia			
ATCC 25015^{T}[b]	100	0.0	100
ATCC 25016	84	2.0	77
KC1394 (ATCC 25017)	86	1.5	83
KC1395 (ATCC 25018)	81	1.5	78
"Philomiragia" bacterium-like			
D2204	83	2.0	77
D7533	73	2.5	72
E384	82	1.5	75
E3596	80	1.0	76
E6588	78	1.5	79
E7485	88	1.0	79
E8869	69	1.5	73
E9088	71	2.0	75
E9923	86	2.0	80
F1853	76	2.0	75
F6055	83	1.0	80
F6820	86	1.0	80
F9017	80	3.5	74
F. novicida KC666^T (Utah 112^T)[b]	51		
F. novicida-like			
D9876	44		
F6168	22		
F. tularensis			
KC1482^T (ATCC 6223^T)[b]	43		
KC1458	35		
E3526	39		
E6702	38		
F6292	37		

[a] Calculated to the nearest 0.5%.
[b] Type strain.

Divergence in related sequences was 1.5 to 2.0% (average, 1.7%). Relatedness in 65°C reactions was 77 to 83% (average, 79%). Relatedness of *Y. philomiragia* ATCC 25015^T to 13 "Philomiragia" bacterium-like strains was 69 to 88% (average, 80%) in 50°C reactions, with 1.0 to 3.5% divergence (average, 1.7%) and 72 to 80% (average, 77%) relatedness in 65°C reactions. *Y. philomiragia* showed 39% average relatedness to the type strain and four other *F. tularensis* strains, the type strain of *F. novicida*, and the two *F. novicida*-like strains (Table 4). In reciprocal reactions, labeled DNA from *F. tularensis* ATCC 6223^T was 41% related to four *Y. philomiragia* strains at 50°C, with 14.5% divergence in related sequences and 18% relatedness at 65°C (Table 5).

Relatedness of *F. tularensis* ATCC 6223^T to four other *F. tularensis* strains was 82% at 50°C (with 0.3% divergence) and 89% at 65°C. Similar high relatedness (93% at 50°C [with 2.0% divergence] and 88% at 65°C) was observed when *F. tularensis* ATCC 6223^T was hybridized with the type strain of *F. novicida* and the two *F. novicida*-like strains (Table 5).

These findings clearly indicate that the 14 "Philomiragia" bacterium-like human isolates belong to the species *Y. philomiragia* and that *Y. philomiragia* is substantially related to *Francisella* species.

TABLE 5. DNA relatedness of *F. tularensis* ATCC 6223^T to strains of *F. tularensis*, *F. novicida*, *Y. philomiragia*, "Philomiragia" bacterium-like, and *F. novicida*-like

Source of unlabeled DNA	% Relatedness (50°C)	% Divergence[a]	% Relatedness (65°C)
F. tularensis			
KC1482^T (ATCC 6223^T)[b]	100	0.0	100
KC1458	72	0.5	75
E3526	95	0.0	98
E6702	67	0.0	83
F6292	92	0.5	91
F. novicida KC666^T (Utah 112^T)[b]	92	1.0	87
F. novicida-like			
D9876	95	2.0	89
F6168	93	2.5	86
Y. philomiragia			
ATCC 25015^{T}[b]	43	14.0	16
ATCC 25017	45	14.0	21
"Philomiragia" bacterium-like			
F6095	38	14.5	18
E7533	39	14.5	16

[a] Calculated to the nearest 0.5%.
[b] Type strain.

The 14 "Philomiragia" bacterium-like human strains were isolated from blood (9 strains), lung biopsy or pleural fluid (3 strains), cerebrospinal fluid (1 strain), and peritoneal fluid (1 strain). Of these 14 patients with *Y. philomiragia* isolated from sterile sites, 5 had chronic granulomatous disease and 5 others had a recent history of near drowning. Two of the four remaining patients had myeloproliferative disorders, and two had no obvious underlying diseases. From the clinical information available for 12 patients, pneumonia was the most common infection noted (5 patients). Fever without a distinct focus of infection (five patients), meningitis, and peritonitis were also reported.

The two human *F. novicida*-like strains were isolated from a cervical lymph node in a 26-year-old man diagnosed as having tularemia and from the blood of a 52-year-old man diagnosed as having hepatitis.

Case summaries of patients with the two human isolates of *F. novicida*-like bacterium. Case 1. A 26-year-old man from Louisiana presented with a 3-week history of a tender, enlarging lump on the left side of his neck. He denied other symptoms and had no underlying diseases. He worked setting up oil rigs and had worked on a farm loading hay several months before hospital admission. He specifically denied any contact with rabbits. Physical examination revealed a 2-by-2-by-1-cm node on the left side of the neck. Complete blood count, serum electrolytes, liver, and renal function tests were within normal limits. Biopsy of the lymph node showed necrotizing granulomas, and a *F. novicida*-like bacterium was grown from the tissue. The patient was discharged after excision of the node and given a 2-week course of tetracycline.

Case 2. A 52-year-old man with a history of alcoholism and chronic peptic ulcer disease was admitted to the hospital after 3 days of dizziness, nausea, vomiting, and fever. On admission, he had a temperature of 103.4°F (39.7°C), a systolic blood pressure of 80 mm Hg, and a pulse of 160/min. Physical examination revealed an enlarged liver (with an edge 8 cm below the costal margin) and right upper quadrant tenderness. The leukocyte count was 7,300/mm^3 with 16% band forms. Alkaline phosphatase was 111 U/liter (normal, 25 to 95 μ/liter), serum glutamic oxalacetic transaminase was 89 U/liter (normal, 7 to 40 U/liter), serum albumin was 2.7 mg/dl (normal, 3.5 to 5.0 mg/dl), and total bilirubin was within normal limits. Six of six blood cultures collected from different sites over a 24-h period grew an *F. novicida*-like bacterium. The patient was begun on intravenous cefoxitin upon admission, and fever subsided over 3 days. He was discharged on hospital day 6 in fair condition.

DISCUSSION

DNA relatedness and biochemical data showed conclusively that the two *F. novicida*-like strains are *F. novicida* and, confirming the DNA relatedness data of Ritter and Gerloff (25) and the transformation studies of Tyeryar and Lawton (29), left no doubt that *F. novicida* and *F. tularensis* are subjective synonyms (different type strains) for the same genetically defined species. *F. tularensis* was differentiated from the single *F. novicida* strain on the basis of its lack of acid production from sucrose, its generally more fastidious growth requirements, and its pathogenicity for humans (10). Strains of the two recognized biogroups (biovars) of *F. tularensis* (biogroups tularensis and palaeartica) differ in biochemistry, pathogenicity, and host range (10, 21). We now know that *F. novicida* is pathogenic for humans.

The clinical and epidemiological features of case 1 resemble classic glandular tularemia (2) or ulceroglandular tularemia without ulcer (11), which may appear as an isolated enlarged lymph node without other obvious symptoms (11). The patient was an otherwise healthy individual with a history of outdoor work, similar to many patients with ulceroglandular tularemia. The second case was more characteristic of typhoidal tularemia. The patient was an alcoholic with poor nutritional status on admission, as were three of four patients with *F. tularensis* bacteremia recently described by Provenza et al. (24). Thus, both cases in which *F. novicida* has been isolated from normally sterile sites in humans are consistent with the previously described epidemiological and clinical picture of disease caused by *F. tularensis*.

In view of genetic similarity and apparently similar pathogenicity, there is no justification for maintaining *F. tularensis* and *F. novicida* as separate species. Since *F. tularensis* was the first to be described (17), it has priority over *F. novicida* (15). We propose that *F. novicida* be included in *F. tularensis* as a third biogroup, *F. tularensis* biogroup novicida. Biochemical characteristics of the three strains of *F. tularensis* biogroup novicida are given in Tables 2 and 6.

TABLE 6. Presumptive identification of *F. philomiragia*, *F. tularensis* biogroup novicida, *F. tularensis* biogroup tularensis, and *F. tularensis* biogroup palaeartica[a]

Test or substrate	*F. philomiragia* (16 strains)	*F. tularensis* biogroup novicida (3 strains)	*F. tularensis* biogroup tularensis (69 strains)[b]	*F. tularensis* biogroup palaeartica (43 strains)[b]
Gram-negative small coccoid forms	100	100	100	100
Aerobic growth	100	100	100	100
Growth on MacConkey agar	14W (14W)	(66W)	6W	0
Oxidase, Kovacs	100	0	0	0
Triple sugar iron agar				
Acid slant	0	0	0	0
Acid butt	0	0	0	0
H_2S (slant or butt)	64W (36)	0	0	0
Growth in:				
Nutrient broth, 0% NaCl	0	0	10	0
Nutrient broth, 6% NaCl	86	66	0	0
Urease	0	0	0	0
Nitrate reduction	0	0	0	0
Acid production from:				
D-Glucose	57W (43W)[c]	(100W)	80, 12W, (6), (1W)[d]	33, 46W, (9), (12W)
Sucrose	57W (43W)[c]	100W	0	0
Maltose	57W (43W)[c]	66W	NT	NT
Glycerol	NT	NT	43, 28W, (17), (12W)[e]	0
Motility	0	0	0	0
Gelatin hydrolysis	75	0	0	0

[a] Test results are given as percent positive after 24 to 48 h; ( ), reaction delayed 3 to 7 days; W, weak reaction; NT, not tested.
[b] Cysteine or cystine usually required for growth. Not all strains were tested in every test.
[c] Difco oxidation-fermentation basal medium used for assaying acid production from carbohydrates for *F. philomiragia* and *F. tularensis* biogroup novicida.
[d] Cysteine agar (carbohydrate base) (6) used for *F. tularensis* biogroup tularensis and *F. tularensis* biogroup palaeartica.
[e] The possession of a citrulline ureidase system also distinguishes *F. tularensis* biovar tularensis from the less virulent *F. tularensis* biovar palaeartica (10).

The substantial genetic relatedness of *Y. philomiragia* to *Francisella* species confirms the conclusion, based on previous biochemical and DNA relatedness results, that *Y. philomiragia* should not be in the genus *Yersinia* and confirms the present biochemical results and cellular fatty acid analyses which indicate that the "Philomiragia" bacterium should be classified in the genus *Francisella*. We propose the name *Francisella philomiragia* comb. nov.

Description of *F. philomiragia* comb. nov. *Francisella philomiragia* (Jensen, Owen, and Jellison 1969) comb. nov. (philo.mi.ra'gi.a. Gr. adj. *philos* loving; M. L. n. *miragia* plural of Latinized English word *mirage*; *philomiragia* loving mirages, because of the mirages that are seen in the area where the isolations of the first strains of this species were made) (1).

The species conforms in part to the description of the genus *Francisella* (10). It must be noted that *F. philomiragia* is oxidase negative (or sometimes weakly positive) by the cytochrome oxidase test (13, 30) but is oxidase positive by the Kovacs modification (Tables 2 and 6). *F. philomiragia* grows well on HIA, HIAB, buffered charcoal-yeast extract agar with and without cysteine, and other ordinary laboratory media. *F. philomiragia* is less fastidious than *F. tularensis*. Colonies are semitranslucent, smooth, glossy, punctate to 0.5 mm in diameter, with entire edges after 24 h. Cells do not appear quite as small as those of *F. tularensis*. H_2S is produced in triple sugar iron agar. Gelatin is usually hydrolyzed. Sucrose is utilized with production of acid but not gas (usually weakly). Further biochemical reactions are shown in Table 2, and tests used in presumptively identifying *F. philomiragia* and differentiating it from other *Francisella* species are shown in Table 6. The cellular fatty acid profile is characteristic of the genus *Francisella* (Table 3).

F. philomiragia has been isolated from water, muskrats, and humans. *F. philomiragia* appears to be a rarely occuring but serious human pathogen. The predilection of *F. philomiragia* for causing disease in two specific risk groups (chronic granulomatous disease patients and near-drowning victims) highlights major differences in the epidemiology of infection with this organism and with *F. tularensis*. The most common risk factors for tularemia in the United States are contact with infected animals and a history of tick bite (2). Eighty-six percent of persons infected with *F. philomiragia* had an underlying immunosuppressive illness or near-drowning, while such persons constituted a minority of those with tularemia, suggesting that *F. tularensis* is the more virulent pathogen. Nevertheless, infection with *F. philomiragia* resulted in the death of 1 of the 14 patients and clinical illness, including pneumonia and bacteremia without a known source, in most of the remaining patients (J. D. Wenger, D. G. Hollis, R. E. Weaver, C. N. Baker, G. R. Brown, D. J. Brenner, and C. V. Broome, submitted for publication).

G+C content is 33 to 34 mol%.

The type strain is ATCC 25015. Its G+C content is 34 mol%.

ACKNOWLEDGMENTS

We thank Carolyn N. Baker for the antimicrobial susceptibility testing and William F. Bibb for the fluorescent-antibody tests.

LITERATURE CITED

1. **Bercovier, H., and H. H. Mollaret.** 1984. Genus XIV. *Yersinia* Van Loghem 1944, 15[AL], p. 498–506. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
2. **Boyce, J. M.** 1985. *Francisella tularensis* (tularemia), p. 1290–1294. *In* G. L. Mendell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious diseases, 2nd ed. John Wiley & Sons, Inc., New York.
3. **Breed, R. S., E. F. Lessel, and E. H. Clise.** 1957. Genus I. *Pasteurella* Trevisan, 1887 p. 395–402. *In* R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore.
4. **Brenner, D. J., A. C. McWhorter, J. K. Leete Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15:**1133–1140.
5. **Brenner, D. J., A. G. Steigerwalt, R. E. Weaver, J. E. McDade, J. C. Feeley, and M. Mandel.** 1978. Classification of the Legionnaires' disease bacterium: an interim report. Curr. Microbiol. **1:**71–75.
6. **Clark, W. A., D. G. Hollis, R. E. Weaver, and P. Riley.** 1984. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria. Centers for Disease Control, Atlanta.
7. **Dees, S. B., C. W. Moss, D. G. Hollis, and R. E. Weaver.** 1986. Chemical characterization of *Flavobacterium odoratum*, *Flavobacterium breve*, and *Flavobacterium*-like groups IIe, IIh, and IIf. J. Clin. Microbiol. **23:**267–273.
8. **Dorofe'ev, K. A.** 1947. Classification of the causative agent of tularemia. Symp. Res. Works Inst. Epidemiol. Mikrobiol. Chita **1:**170–180.
9. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
10. **Eigelsbach, H. T., and V. G. McGann.** 1984. Genus *Francisella* Dorofe'ev 1947, 176[AL], p. 394–399. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
11. **Evans, M. E., D. W. Gregory, W. Schaffner, and Z. A. McGee.** 1985. Tularemia: a 30-year experience with 88 cases. Medicine (Baltimore) **64:**251–269.
12. **Jantzen, K., B. P. Berdal, and T. Omland.** 1979. Cellular fatty acid composition of *Francisella tularensis*. J. Clin. Microbiol. **10:**928–930.
13. **Jensen, W. I., C. R. Owen, and W. J. Jellison.** 1969. *Yersinia philomiragia* sp. n., a new member of the *Pasteurella* group of bacteria, naturally pathogenic for the muskrat (*Ondatra zibethica*). J. Bacteriol. **100:**1237–1241.
14. **Lambert, M. A., C. M. Patton, T. J. Barrett, and C. W. Moss.** 1987. Differentiation of *Campylobacter* and *Campylobacter*-like organisms by cellular fatty acid composition. J. Clin. Microbiol. **25:**706–713.
15. **Larson, C. L., W. Wicht, and W. L. Jellison.** 1955. An organism resembling *P. tularensis* from water. Public Health Rep. **70:** 253–258.
16. **Marmur, J., and P. Doty.** 1962. Determination of base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5:**109–118.
17. **McCoy, G. W., and C. W. Chapin.** 1912. Further observations on a plague-like disease of rodents with a preliminary note on the causative agent, *Bacterium tularense*. J. Infect. Dis. **10:** 61–72.
18. **Moss, C. W., A. Kai, M. A. Lambert, and C. M. Patton.** 1984. Isoprenoid quinone content and cellular fatty acid composition of *Campylobacter* species. J. Clin. Microbiol. **19:**772–776.
19. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
20. **O'Hara, S., T. Sato, and M. Homma.** 1974. Serological studies on *Francisella tularensis*, *Francisella novicida*, *Yersinia philomiragia*, and *Brucella abortus*. Int. J. Syst. Bacteriol. **24:** 191–196.
21. **Olsufiev, N. G., O. S. Emelyanova, and T. N. Dunaeva.** 1959. Comparative studies of strains of *B. tularense* in the Old and New World and their taxonomy. J. Hyg. Epidemiol. Microbiol.

Immunol. (Prague) **3**:138–149.

22. **Owen, C. R.** 1974. Genus *Francisella* Dorofe'ev 1947, 176, p. 283–285. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
23. **Owen, C. R., E. O. Buker, W. L. Jellison, D. B. Lackman, and J. F. Bell.** 1964. Comparative studies of *Francisella tularensis* and *Francisella novicida*. J. Bacteriol. **87**:676–683.
24. **Provenza, J. M., S. A. Klotz, and R. L. Penn.** 1986. Isolation of *Francisella tularensis* from blood. J. Clin. Microbiol. **24**:453–455.
25. **Ritter, D. B., and R. K. Gerloff.** 1966. Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. J. Bacteriol. **92**:1838–1839.
26. **Schoenknecht, F. D., L. D. Sabath, and C. Thornsberry.** 1985. Susceptibility tests: special tests, p. 1000–1008. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
27. **Seger, R. A., D. G. Hollis, R. E. Weaver, and W. H. Hitzig.** 1982. Chronic granulomatous disease: fatal septicemia caused by an unnamed gram-negative bacterium. J. Clin. Microbiol. **16**:821–825.
28. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.).** 1980. Approved lists of bacterial names, p. 419. American Society for Microbiology, Washington, D.C.
29. **Tyeryar, F. J., Jr., and W. D. Lawton.** 1969. Transformation in *Pasteurella novicida*. J. Bacteriol. **100**:1112–1113.
30. **Ursing, J., A. G. Steigerwalt, and D. J. Brenner.** 1980. Lack of genetic relatedness between *Yersinia philomiragia* (the "Philomiragia" bacterium) and *Yersinia* species. Curr. Microbiol. **4**:231–233.
31. **Wilson, G. S., and A. A. Miles.** 1964. *Brucella tularensis*, p. 1013–1014. *In* Topley and Wilson's principles of bacteriology and immunity, 5th ed., vol. 1. The Williams & Wilkins Co., Baltimore.